DEPARTMENT OF HEALTH AND HUMAN SERVICES

NOTE TO THE FILE
April 21, 1998

Subject: BNF0055 - Glufosinate-tolerant Soybean Lines

Keywords:
Soybean, *Glycine max*, Glufosinate, Liberty®, Liberty Link® soybeans, phosphinothricine acetyltransferase (PAT), *pat* gene, , *Streptomyces viridochromogenes*, beta-lactamase (*bla*) gene

Background
The new soybean varieties that are the subject of this consultation contain the *pat* gene from *Streptomyces viridochromogenes*. The *pat* gene encodes the phosphinothricin acetyltransferase (PAT) enzyme, which confers resistance to glufosinate ammonium herbicides. AgrEvo first provided some background information on the new soybean varieties (derived from events A2704-12 and A5547-127) on March 21, 1998. AgrEvo then submitted a full summary of safety and nutritional assessment of the new soybean varieties on March 31, 1998. AgrEvo noted that the mode of action of the enzyme phosphinothricin acetyltransferase and information on its safety have been submitted to FDA as part of previous food and feed safety assessment of other glufosinate-tolerant crops.

Intended Effect and Food/Feed Use
The intended technical effect of the genetic modification of the new soybean lines is to confer tolerance to the herbicide glufosinate. AgrEvo notes that the food and feed uses of the new soybean varieties are no different than those of varieties currently on the market. Soybeans or processed products derived from soybeans are used for both human and animal food. In addition to oil for human consumption and defatted meal for animal feed, whole soybeans are used to produce such items as soy sprouts, baked soybeans, roasted soybeans, full fat soy flour and traditional soy foods such as miso, soy milk, soy sauce and tofu. Soybean protein products also have food and feed uses.

Molecular Alterations and Characterization
Both new soybean varieties were produced by the particle bombardment method of generating transformants using a plasmid with a pUC backbone and containing the *pat* gene with the Cauliflower Mosaic Virus 35S promoter and terminator. The plasmid was linearized using a restriction enzyme prior to use in the transformation process in order to destroy the beta-lactamase (*bla*) gene found in the plasmid backbone.

The *pat* gene that has been introduced into the new soybean varieties is a synthetic version of the *S. viridochromogenes* gene. Since the bacterial *pat* gene has a high G:C content, which is atypical for plants, AgrEvo synthesized a gene with a modified nucleotide sequence using codons preferred by plants. AgrEvo notes, however, that while the nucleotide sequence has been altered, the amino acid sequence of the enzyme remains unchanged.

AgrEvo states that subsequent analysis of the transformants shows that two copies of the *pat* gene integrated into the genome of transformant A2704-12 in a head-to-tail configuration and that one copy of 3' *bla* sequences and one copy of 5' *bla* sequences are integrated in between the two *pat* genes. The integrated parts of the *bla* gene do not reconstitute an intact *bla* gene because the sequences integrated into the genome in an inverted orientation with respect to each other. In transformant A5547-127, one copy of 3' *bla* sequences, and one copy of 5' *bla* sequences are integrated downstream, and upstream, respectively, of a single copy of the *pat* gene that integrated into the plant genome. The 5' and 3' *bla* sequences do not reconstitute an intact *bla* gene because of the intervening *pat* gene. For both transformation events, examination of progeny shows that the *pat* locus is inherited in Mendelian fashion.

Safety of Expressed Protein

Only one protein, namely, the phosphinothricin acetyltransferase enzyme is expressed in the new soybean varieties. The *bla* gene is not expressed; expression is not expected because the gene is not present in the soybean genome in an intact form. Expression would not be expected even if an intact gene was present because a bacterial promoter was used with the gene. In any case, AgrEvo stated that Northern blot analysis it conducted showed that no *bla* messenger RNA is made in the transgenic soybeans.

AgrEvo states that it measured PAT protein levels in whole and processed plant fractions using enzyme-linked immunosorbent assays (ELISA) in order to determine whether the PAT protein would be a major constituent in human or livestock diets. AgrEvo states that using the largest amounts of PAT detected and the smallest level of crude protein reported in the literature for forage, hay and seed, PAT protein represents 0.0031%, 0.0015%, and 0.0005% of the crude protein in forage, hay and seed in event A2704-12, respectively. The corresponding percentages for event A5547-127 are 0.0174%, 0.0034%, and 0.0031% for forage, hay, and seed respectively.

AgrEvo states that, as expected, there was no PAT protein detected in refined oil, food grade oil, and crude lecithin for both events. No PAT activity was detected in the meal fractions or the soy isolates from event A2704-12 although the protein was detected in the hull fraction. For event A5547-127, immunoreactive PAT protein was detected in all processed fractions except refined oil, food grade oil, and crude lecithin.

AgrEvo notes that ELISA detects both active and inactive PAT protein and that, while activity assays were not done to determine if any of the immunoreactive PAT is enzymatically active, processing temperatures are sufficiently high to inactivate the enzyme. AgrEvo adds that regardless of the level present in whole and processed fractions, PAT is not likely to be toxic or allergenic. AgrEvo notes that biochemical characterization, and allergenic and toxicity potential of the PAT protein have been addressed in previous submissions to FDA (BNFs 0023, 0029, and 0046). In addition, AgrEvo states that PAT protein showed no evidence of toxicity in rats fed the protein at dietary concentrations of 50,000 ppm for 14

days. AgrEvo also fed broilers diets prepared from the two new soybean varieties as well as nontransgenic soybean and reported that evaluation of body weight, body weight gain, feed intake and carcass characteristics showed no differences between chickens fed diets using seed from A2704-12 or A5547-127 and commercial varieties.

Compositional Analysis

Antinutrients

AgrEvo analyzed for stachyose, raffinose and phytic acid in seed, and for trypsin inhibitor and lectins in seed, non-toasted soy meal, and toasted soy meal. AgrEvo also measured phytoestrogen levels in seed, as well as non-toasted and toasted soy meal (AgrEvo noted that although phytoestrogens such as the isoflavones daidzein, genistein, and glycitein have been implicated in adverse effects on the reproductive system in animals fed large amounts of soybean meal, they are not universally accepted as anti-nutrients, and that they have also been reported as having beneficial anti-cancer effects). AgrEvo stated that the antinutrient content of the new soybeans does not differ significantly from that of non-transgenic soybeans.

Allergens

AgrEvo also analyzed whether the genetic modification might have altered the allergenic potential of seed derived from the new soybean varieties. AgrEvo screened soybean seed extracts from lines derived form both A2704-12 and A5547-127 events as well as their non-transgenic counterparts against a panel of sera from 16 soy-allergic individuals using Radioallergosorbent Test (RAST). In other studies, pooled sera were used in RAST inhibition assays and in immunoblotting. AgrEvo reports that the results of these studies demonstrate that there is no qualitative or quantitative difference in endogenous soybean allergen content between the two transgenic soybean varieties and their non-transgenic counterparts.

Nutrients

AgrEvo conducted the following analyses for lines derived from transformation events A2704-12 and A5547-127: 1) proximate analysis (moisture, crude protein, crude fat, ash, acid detergent fiber, neutral detergent fiber, carbohydrate) in hay, forage, seed, hulls, and toasted and non-toasted defatted soy meal; 2) minerals (calcium, phosphorous, and potassium) in seed; 3) fatty acid analysis in seed and food grade oil; and 4) amino acid analysis in seed, soy isolates, and non-toasted as well as toasted soy meal.

AgrEvo reported that there were no nutritionally significant differences in proximates or mineral contents between the new soybean varieties and current commercial varieties and that values obtained were similar to values established for soybeans. In addition, no statistically significant differences were observed for amino acid levels between soybeans derived from event A5547-127 and non-transgenic soybeans. Statistical differences were seen in the levels of some amino acids between soybeans derived from event A2704-12 and non-transgenic counterpart with the levels in the new variety being higher. AgrEvo states that although

differences were noted, the amino acid profiles for both soybean varieties and the non-transgenic varieties were qualitatively and quantitatively similar to those reported by the US Department of Agriculture (USDA) for soybeans with glutamic acid being present at the highest level and tryptophan being present at the lowest level. Similarly, when the fatty acid profile of oil derived from the new soybean varieties was compared to the fatty acid profile of oil derived from non-transgenic soybean, differences were observed for some fatty acids. However, AgrEvo notes that the qualitative and quantitative profiles of total lipids and fatty acids of the transgenic soybeans as well as non-transgenic soybeans are similar to profiles reported for soybeans by USDA.

AgrEvo notes that the statistically significant differences in some amino acids and fatty acids could be due to factors such as growing conditions, maturity at harvest, post harvest storage and processing, and the inherent variability of nutrient content. The differences were not observed consistently in samples obtained from all growing regions. AgrEvo concludes that the differences observed are not meaningful from a nutritional point of view.

Conclusions

AgrEvo has concluded that its transgenic glufosinate-tolerant soybean lines are not materially different in terms of food safety and nutritional profile from soybean varieties currently on the market. At this time, based on AgrEvo's description of its data and analyses, the Agency considers AgrEvo's consultation on soybean lines from transformation events A2704-12 and A5547-127 to be complete.

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